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Exploratory study of correlation between lipid profile and bone mineral density in postmenopausal women (Campinas Hospital);

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Janet Carper / On behalf of
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County of Fairfax
Commonwealth of Virginia
Subscribed and sworn before me
this 20th day of June , 2005,

Lidia Terziotti, Notary Public
My commission expires September 30, 2007

Exploratory study of correlation between lipid profile and bone mineral density in postmenopausal women (Campinas Hospital)

Association between profile and bone mineral density in post-menopausal women

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[Please see original article for abstract in English]

Introduction

With the significant increase in the life expectancy of women, and consequently of chronic diseases such as cardiovascular disease and osteoporosis, many health professionals have ordered lipid profiles and bone mass measurements almost routinely when caring for menopausal women.

However, these supplementary tests are usually burdensome for women and even for public health services as they are normally done every year in all women seen for menopausal issues. In Campinas, the estimated dollar cost of the complete plasma lipid profile is between US$35 and US$50 and between US$75 and US$100 for the bone mineral density test. This does not include the women's costs for transportation and food every day they come to the hospital for testing.

Bagbozan et al. (1993) and Soo et al. (1993) studied, in menopausal women, the relationship of total cholesterol and LDL cholesterol to bone density scans of the lumbar spine. These authors suggest that women with elevated total cholesterol and LDL cholesterol, namely at risk of cardiovascular disease, also had a low lumbar spine bone mass. No other articles on this correlation were found in the literature, therefore this new finding called for further clinical research.

These results, together with the idea that the lipid profile might be a replacement for the bone density scan in screening for osteoporosis in menopausal women, were the motivation for this study which aims to simplify the routine workup when caring for menopausal women, thus cutting costs for the health system and the patient while preserving safety and quality of care.

Patients and Methods

The study group consisted of 72 menopausal women aged 41 to 65, seen at the menopause out-patient clinic of the CAISM/Unicamp (Integral Women's Health Care Center, State University of Campinas) in 1995, a year in which 400 women were seen at the clinic. All the patients had been amenorrheic for over a year and had not used hormone replacement therapy during or prior to that period. The study excluded patients who had had hysterectomies or had any chronic disease such as high blood pressure, diabetes mellitus, joint disease, thyroid disease (hyperthyroidism or hypothyroidism), heart disease (arrhythmia and ischemia), etc. or malignant neoplasia. These exclusion criteria were used so that the tests for some of these diseases, and the use of hormone replacement therapy, would not influence the result of the lipid profile or bone density scan. There were no refusals, and we finalized screening when the necessary sample size was achieved.

The sample size was calculated on 61 patients, taking 1.16 g/cm2 (Kish, 1965) for the average bone mineral density in the lumbar spine of postmenopausal women in the population at large, with a standard deviation (SD) of 0.16 g/cm2, alpha error 0.05, and taking 0.04 as the desired difference between the average bone mineral density in postmenopausal women and in the general population (Souza & Schneider, 1993).

For the lipid profile, we drew 10 mL of blood with disposable needle and syringe after a total 14-hour fast in the collecting room of the Unicamp Hospital. The blood was then analyzed by technicians and checked by the responsible clinical pathologist, so that a duplicate set of findings was issued according to the usual practice by the Unicamp Hospital Clinical Pathology Laboratory.

The bone mass was measured by a bone density scan using a Lunar DPX by specialists in the Unicamp Hospital Nuclear Medicine Department, producing a computer printout. Three femoral sites (femoral neck, Wards triangle, and trochanter) plus the lumbar spine (using the space between L2 and L4) were chosen for the bone mineral scan.

The data were analyzed in two steps. First, the difference between the average bone mineral densities at the various sites was analyzed according to stratified lipid profile values by the Student's T test and ANOVA. We then did a multiple regression analysis using the lumbar spine (L2-L4), femoral neck, Wards triangle, and trochanter bone mineral density as the dependent variable, referring to the four models developed, and the lipid profile represented by total cholesterol and Castelli's Index 2 (Cox, 1970) as the independent variable.

In the second step, we validated the lipid profile as a diagnostic test for osteoporosis, considering the bone density scan as the gold standard. We calculated the sensitivity and specificity and plotted the receiver operator characteristic (ROC) curve, and determined the cutoff point for each of the variables of the lipid profile analyzed. Tests of good discriminatory power were concentrated on the upper left corner of the ROC curve, and lower discriminatory power tests had curves closer to the diagonal (Fletcher et al., 1991).

RESULTS

Characterization of population

The age of the 72 patients studied ranged between 41 and 65, averaging 52 (SD ± 4.74). The average age for occurrence of menopause was 48 (SD ± 3.34) so that, for over half the patients evaluated, menopause occurred between the ages of 51 and 55. The average time since menopause was four years, and for the majority of the menopausal women it was less than two years (Tables 1, 2, and 3).

The average values of the lipid profile indicators were within the normal range according to reference values, with the exception of total cholesterol and HDL cholesterol. Total cholesterol showed an average of 213 mg%, which can be considered higher than standard

normal values. The HDL cholesterol showed average values of 59.67 mg%, considered to be below the normal limit.

With respect to the average bone mineral density values in the various areas of the femur and lumbar spine, it was observed that the site of greatest density was the lumbar spine, followed by the femoral neck, Wards triangle, and the trochanter.

The percent bone mineral density distribution according to the diagnosis of normal, osteopenia, and osteoporosis showed that the majority of patients were in the normal range in the femoral neck, Wards triangle, and trochanter but over half of them had alterations in the lumbar spine (Table 4).

Correlation between lipid profile and bone density

When we correlated the lipid profile with bone mineral density, we saw that the lowest bone mineral density values were for the patients with average plasma cholesterol levels that were greater than or equal to 240 mg%. These differences were not statistically significant (Table 5).

For HDL cholesterol, the lowest bone mineral density was observed for the patient group with average HDL cholesterol levels greater than or equal to 80 mg%, with a statistically significant difference for all sites (Table 6).

The average bone mineral density in the femur and lumbar spine was greater in the patient group with an LDL cholesterol between 130 and 159 mg% and less in the group with an LDL cholesterol greater than or equal to 159 mg%. These differences were not statistically significant (Table 7).

In the correlation between bone mineral density at the various sites and the total cholesterol to HDL cholesterol ratio (Castelli's Index 1), we observed a higher average bone mineral density in the patient group with a Castelli's Index 1 greater than or equal to 4.4; however, these differences were not statistically significant (Table 8).

We noted greater bone mineral density averages in the various sites of the femur and lumbar spine in women with Castelli's Index 2 (LCL cholesterol to HDL cholesterol ratio) greater than 3.2; however, these differences were not significant (Table 9).

Multiple linear regression showed a correlation between total cholesterol with values above 240 mg% and of Castelli's Index2 with bone mineral density in the femoral neck, Wards triangle, and trochanter. Total cholesterol values over 240 mg% were associated with less bone mineral density in these sites, which is a reverse correlation. The Castelli Index 2 levels showed a direct correlation, i.e. the higher the average values, the greater the bone mineral density. The model using average bone mineral density values in the lumbar spine showed no statistically significant correlation with the total plasma cholesterol levels above 240 mg% and with Castelli's Index 2 (Table 10).

When we analyze the stratified total cholesterol values as indicators of decreased bone mineral density in the femoral neck and lumbar spine, using the bone density scan as the gold standard, we see that the best cutoff point for the two sites is 210 mg% (Figure 1). Even at the best cutoff point, sensitivity and specificity were low, both for the femoral neck and for the lumbar spine.

The stratified HDL cholesterol values as indicators of decreased bone mineral density in the femoral neck and lumbar spine also had low sensitivity and specificity, even for the best cutoff point, which was 50 mg% for both sites (Figure 2).

Also, the stratified LDL cholesterol values as indicators of decreased bone mineral density in the femoral neck and lumbar spine had low sensitivity and specificity for the best cutoff point (110 mg%) for both sites (Figure 3).

Discussion

The main purpose of this study was to assess any correlation between some lipid profile variables and bone mineral density in menopausal women. It is an attempt to see whether women at a higher risk of developing cardiovascular disease also have a higher risk for osteoporosis. We concluded that the lipid profile is not a good indicator of bone density, as we saw contradictory results. The results show that high total plasma cholesterol values are associated with lower BONE MINERAL DENSITY while high low- and high-density lipoprotein ratios, i.e. risk for cardiovascular disease, were correlated with higher bone mineral density.

It must also be borne in mind that, for every lipid profile category value evaluated, low sensitivity and specificity in relation to BONE MINERAL DENSITY were found, for a given value. In other words, the ability of the levels of total cholesterol and some of its fractions to predict the occurrence of lower BONE MINERAL DENSITY values was very low. Its ability to diagnose the existence of decreased bone mass was also low. The cutoff point used for the lipid profile variables in calculating its sensitivity and specificity as a bone mass indicator was obtained by the ROC curve, which shows the ratio between the sensitivity and specificity of the various values in the lipid profile categories. The cutoff point value with sensitivity and specificity well-balanced is the one closest to the upper left corner. The straighter the curve and the closer it is to the diagonal, the worse the test performance.

It might be thought that one important factor in the inconsistency of the study results could be the differences in the prevalence of lipid profile alterations, in relation both to national and to international studies. In prior studies on out-patient menopausal women at CAISM-Unicamp, it was observed that total cholesterol was normal in 90.4% of the 235 cases evaluated, with LDL cholesterol, HDL cholesterol, and LDL cholesterol [*sic* – presumably one of these values was meant to be "total cholesterol" – translator] unaltered in 99.1%, 96.8%, and 86.3% of the women respectively (Pinto Neto et al., 1991), a different situation from that observed by other authors conducting population studies in other areas, including the São Paolo metropolitan area, which showed that about 15% of the total serum cholesterol values were above normal (Martins, 1989; Bagbozan et al., 1993; Soo et al., 1993). Another point that should also be borne in mind refers to the sample variation, which may have occurred in this study.

With respect to osteoporosis, we should recall that the patient group in this study consisted mainly of women under 55 and recently menopausal, with few risk factors for this pathology; thus, one would expect a high percentage of patients with a normal bone mineral density at the various sites evaluated. This may also account for the differences in the results observed from the Soo et al. (1993) and Bagbozan et al. (1993) studies, as well as differences in the characteristics of the population studied.

It is also important to emphasize one unexpected datum that occurred in this study. An inverse correlation was observed between HDL cholesterol and bone mass when the difference between bone mineral density averages was tested at the various sites and their stratified values varied. A risk profile for cardiovascular diseases showed very low HDL cholesterol levels, concomitantly with an elevation in LDL cholesterol and total cholesterol levels. This was an unexpected result, as there was no correlation with the other lipid profile fractions when the same statistical calculation was used, and was even more unexpected for being a reverse correlation, i.e., the better the lipid profile (high HDL cholesterol levels) the lower the BONE MINERAL DENSITY values observed.

In the multiple regression analysis, a direct correlation was also observed between Castelli's Index 2 and bone mass. As Castelli's Index 2 represents the LDL/HDL cholesterol ratio, one would expect to find high values of these

associated with lower BONE MINERAL DENSITY values – which did not happen. So, this result too was inconsistent. We did not include certain possibly confounding variables in the regression model, such as age, as the great majority of the patients were in the 46 to 55 age bracket, so that the patient group was not heterogeneous for this variable. The same point could also be made for post-menopausal time: the average time since menopause was four years, and was less than six years for the great majority of the menopausal women.

Finally, we understand that the care of menopausal women in the Brazilian context where primary medical care facilities – responsible for treating most of the population – are insufficiently equipped for diagnosis and inadequately trained in the various therapeutic perspectives, must be based on prevention of all the diseases afflicting individuals in this age group, prioritizing the prevalence of identifiable risk factors. Despite the role played by the lipid profile in the occurrence of cardiovascular diseases, we do not recommend that it be quantified routinely. Likewise, for osteoporosis, we do not believe bone scans should be done routinely (Cummings & Black, 1986). Prevention of cardiovascular disease and osteoporosis should center on recognizing risk factors and changing lifestyles (Paiva et al., 1995; Fernandes & Pereira Filho, 1995; Lobo, 1994; Marques Neto & Lederman, 1995). We also believe, among other points, that failure to confirm a hypothesis does not mean that resources should not be spent on this line of research. Indeed, due to the importance of the matter, new studies should be done.

Table 1
Average values for age, age at menopause, and years post- menopause (n = 72).

Variables	Minimum	Maximum	X	SD
Age	41	65	52.14	4.74
Age at onset of menopause	38	56	48.12	3.45
Duration of menopause	01	17	4.01	3.22

Table 2
Percent distribution of women by age bracket

Age (years)	n	%
41 to 45	3	4.2
46 to 50	25	34.7
51 to 55	30	41.7
56 to 60	8	11.2
61 to 65	6	8.4
Total	72	100

Table 3
Percent distribution of women by number of years post-menopause

Time post-menopause (years)	n	%
Up to 2	33	45.8
2 to 5	24	33.4
≥ 6	15	20.8
Total	72	100.0

Table 4
Percent distribution of bone mineral density by diagnosis: normal, osteopenia, or osteoporosis (n = 73)

Site	Normal		Osteopenia		Osteoporosis	
Femoral neck	50	68.5	22	30	1	1.4
Wards triangle	41	56.1	29	39.7	3	4.1
Trochanter	56	76.7	17	23.3	0	0
Lumbar spine	35	47.9	28	38.5	10	13.7

Table 5
Average and SD of bone mineral density at various sites, according to stratified total cholesterol values

	Total cholesterol (mg%)						
Site	Up to 200 (n=28)		200 to 239 (n=28)		≥ 240 (n=16)		p*
	X	SD	X	SD	X	SD	
Femoral neck	0.944	0.135	0.961	0.150	0.879	0.129	0.169
Wards triangle	0.808	0.145	0.837	0.162	0.734	0.166	0.089
Trochanter	0.769	0.141	0.802	0.149	0.732	0.111	0.154
Lumbar spine	1.083	0.159	1.048	0.307	1.049	0.179	0.735

*ANOVA

Table 6
Average and SD of bone mineral density at various sites, according to stratified HDL cholesterol values

	HDL cholesterol (mg%)				
Site	< 80 (n=66)		≥ 80 (n=6)		p*
	X	SD	X	SD	
Femoral neck	0.951	0.134	0.764	0.095	0.001
Wards triangle	0.819	0.153	0.614	0.104	0.002
Trochanter	0.789	0.133	0.596	0.067	0.001
Lumbar spine	1.079	0.228	0.874	0.157	0.006

*Student's t test

Table 7
Average and SD of bone mineral density at various sites, according to stratified LDL cholesterol values

	LDL cholesterol (mg%)						
Site	≤ 130 (n=46)		130-159 (n=18)		≥ 159 (n=8)		p*
	X	SD	X	SD	X	SD	
Femoral neck	0.933	0.133	0.968	0.148	0.876	0.165	0.309
Wards triangle	0.794	0.142	0.858	0.168	0.729	0.211	0.129
Trochanter	0.762	0.136	0.812	0.151	0.750	0.133	0.597
Lumbar spine	1.044	0.240	1.129	0.195	1.017	0.299	0.332

*ANOVA

Table 8
Average and SD of bone mineral density at various sites, according to total cholesterol/HDL cholesterol ratio (Castelli's Index 1)

Site	Castelli 1 (n=71)				p*
	< 4.4 (n=57)		≥ 4.4 (n=14)		
	X	SD	X	SD	
Femoral neck	0.917	0.132	1.012	0.158	0.397
Wards triangle	0.783	0.137	0.885	0.217	0.135
Trochanter	0.751	0.124	0.867	0.165	0.168
Lumbar spine	1.063	0.180	1.136	0.232	0.222

*Student's t test

Table 9
Average and SD of bone mineral density at various sites, according to LDL cholesterol/HDL cholesterol ratio (Castelli's Index 2)

Site	Castelli 2 (n=70)				p*
	< 3.2 (n=64)		≥ 3.2 (n=6)		
	X	SD	X	SD	
Femoral neck	0.922	0.132	1.067	0.184	0.272
Wards triangle	0.788	0.145	0.929	0.248	0.062
Trochanter	0.759	0.129	0.895	0.183	0.249
Lumbar spine	1.057	0.178	1.306	1.528	0.749

*Student's t test

Table 10
Lipid profile variables correlated to bone mineral density at sites (multiple regression analysis)

Lipid profile variables	Coefficient	EP* coefficient	p
Neck			
Cholesterol ≥ 240 mg%	-0.126	0.052	0.017
Castelli's Index 2	0.058	0.027	0.034
Constant	0.842	0.055	<0.001
Triangle			
Cholesterol ≥ 240 mg%	-0.146	0.057	0.013
Castelli's Index 2	0.007	0.029	0.011
Constant	0.665	0.061	<0.001
Trochanter			
Cholesterol ≥ 240 mg%	-0.113	0.049	0.026
Castelli's Index 2	0.079	0.026	0.002
Constant	0.622	0.061	<0.001
Lumbar spine			
Cholesterol ≥ 240 mg%	-0.111	0.087	0.025

Castelli's Index 2	0.085	0.045	0.064
Constant	0.927	0.093	<0.001

*EP presumably stands for *erro padrão* (standard error) –translator

Figure 1
ROC curve of total cholesterol as predictor of decreased BMD in the femoral neck and lumbar spine

Lumbar spine
Femoral neck

Sensitivity

specificity

	A	B	C	D	E	F	G	H	I	J
Value of x	0	0	0.009	0	0.12	0.24	0.46	0.67	0.88	0.97
Y – lumbar spine	0	0.05	0.1	0.13	0.21	0.29	0.5	0.68	0.89	1
Y – femoral neck	0	0.04	0.13	0.17	0.26	0.34	0.54	0.78	0.86	1

*Point with 50% sensitivity and 54% specificity closest to the upper left corner of the graph.

Figure 2
ROC curve of HDL cholesterol as predictor of decreased BMD in the femoral neck and lumbar spine

Lumbar spine
Femoral neck

Sensitivity

specificity

	A	B	C	D	E	F	G
Value of x	0	0.04	0.3	0.64	0.79	0.97	0.97
Y – lumbar spine	-	0.08	0.43	0.65	0.81	0.89	0.94
Y – femoral neck	0	0	0.36	0.41	0.68	0.82	1

*Point with 43% sensitivity and 70% specificity closest to the upper left corner of the figure.

Figure 3
ROC curve of LDL cholesterol as predictor of decreased BMD in the femoral neck and lumbar spine

Lumbar spine
Femoral neck

Sensitivity

specificity

	A	B	C	D	E	F	G	H	I
Value of x	0	0	0.03	0.04	0.18	0.38	0.55	0.88	0.97
Y – lumbar spine	-	0.03	0.08	0.13	0.24	0.39	0.62	0.94	1
Y – femoral neck	0	0.04	0.14	0.18	0.23	0.41	0.68	0.95	1

*Point with 62% sensitivity and 45% specificity closest to the upper left corner of the figure

[Please see original article for **References** section]